Three Lupane Derivatives from *Leptospermum scoparium*

Ralf Mayer

Pharmazeutisches Institut der Universität Bonn, Kreuzbergweg 26, D-53115 Bonn, Germany

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Summary

The ether extract of the aerial parts of *Leptospermum scoparium* cultivars yielded a lactone with a 20,29,30- trinorlupane skeleton (**1**). Furthermore, 2α-hydroxyursolic acid (**2**), platanic acid (**3**) and 3β,30-dihydroxy-lup-20(29)-en-28-oic acid (**4**) were isolated.

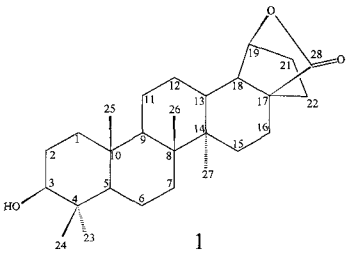
Introduction

The New Zealand medicinal plant *Leptospermum scoparium* Forst. (Myrtaceae) shows numerous varieties and even an intergeneric hybridisation in its natural occurrence[1]. In Europe, *Leptospermum scoparium* is cultivated for medical use and also, mostly hybrids, are available as ornamental plants. Recently, triterpene coumaroyl esters[2a], *C*-methylated flavonoids[2b-4], and common triterpenes[4a] were isolated from wild plant material and cultivars, respectively.

The present paper deals with four minor components of the ether extract of a European *L.s.* cultivar (source **I**); these are detected by TLC comparison also in wild plant material (source **II**) from New Zealand.

Results and Discussion

The ether extract[4a] of *L.s.* cultivars (overground plant parts, source **I**) was submitted to an acetonitrile/chloroform precipitation as described in ref.[5]. CC separation of the sediment yielded **1**, next to β-sitosterol, betulinol, and



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uvaol[4a]. The high-resolved molecular ion peak of **1** at *m/z* 414.3134 gave the molecular formula of $C_{27}H_{42}O_3$, and, taking into account the melting point of 298 °C, a polycyclic trinortriterpene was considered. In addition to the loss of water (base peak) and a methyl group, mass differences of 28 (CO), 44 (CO_2), 45 ($HCOO^{\bullet}$), and 46 (HCOOH) amu are observed. These fragments, along with a very strong 1765 cm^{-1} IR band, gave hints of a five-membered lactone ring. Beneath *m/z* 150, the MS of **1** closely resembles that of betulinic acid (**5**) which is also present in *L.s.*. Also within the ^{13}C NMR data of **1**, close agreement is found with **5**[6] for the sequence C-1 to C-11 (rings A, B, and partly C). The APT features a carbonyl signal at 179.3 ppm as the only sp^2 carbon, thus excluding C-C double bonds. Since **1** presents five methyl group singlets in the ^1H NMR, a 20,29,30-trinorlupane with an additional five-membered lactone ring seemed most likely. Through the correlation of ^{13}C- and ^1H NMR shifts by an HMQC experiment, and along with ^2J- and ^3J-C-H relationships as apparent from HMBC analysis, an *ab initio* deduction of the skeleton succeeded. As against **5**, the carbons 16 and 22 in **1** experience a shielding of about 10 and 8 ppm in the ^{13}C NMR, respectively; likewise, C-13, C-14, and C-15 are slightly affected. These shift effects with regard to **5** are explained by steric interactions of the rigid lactone ring, which is bridging ring E between C-17 and C-19. HMBC correlations of the carbonyl C-28 are observed with 16-H_α, 18-H, 19-H, and 22-H_{AB}. Thus , **1** was identified as 3β,19β-dihydroxy-20,29,30-trinorlupan-28,19-olide. Lactone formation between C-28 and a carbinol at C-19 is further supported by certain ROESY cross peaks (see Figure 1): thus, 19-H interferes sterically with 12-H_β, 13-H, 18-H and 21-H_{AB}. The lack of an isopropenyl side chain as in **5** causes a deshielding of ^{13}C-12 in **1** of about 2 ppm. A proton NMR spectrum of **1** was first taken in $CDCl_3$, but a better resolution of CH_2-AB systems was achieved when recorded in d_6-benzene.

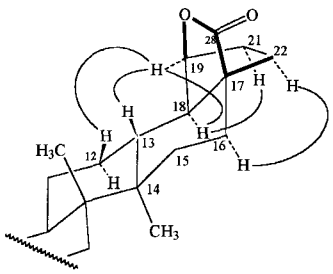

Figure 1. Some ROESY correlations in **1**.

This is clearly the first occurrence of **1** as a natural product; however, **1** had been prepared earlier from 3-*O*-acetylbetulinic acid methyl ester in a five step reaction sequence and characterised mainly by its CD curve[7]; for the first time, its detailed NMR data are given in Tables 1 and 2.

Compounds **2**, **3**, and **4** were isolated from the ether extract (**I**). **2** occurs frequently as in association with ursolic acid (**6**), which is one of the main components of the *Leptospermum scoparium* ether extract. Platanic acid (**3**) was first isolated from *Platanus* hybrids[8], its formation by ozonolysis of **5**-methyl ester[8] was successfully repeated with **5**. Recently, an anti-HIV effect of **3** was reported[9]. **4**, 3β,30-dihydroxy-lup-20(29)-en-28-oic acid, had been isolated from *Relhania genistifolia* in form of its methyl ester[10]. An attempt was

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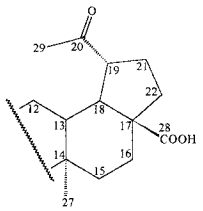

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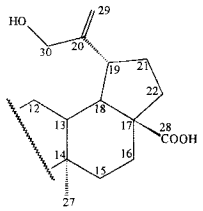

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Table 1: ^{13}C NMR data of compounds **1**, **3**, and **4**.

C No.	**1** ($CDCl_3$)	**3** ($CDCl_3$)	**4** (d_6 -acetone)
1	39.0	38.7	39.5
2	27.5	27.3	28.2
3	79.0	78.9	78.5
4	38.9	38.9	39.6
5	55.5	55.3	56.2
6	18.3	18.3	19.0
7	34.3	34.2	35.1
8	40.7	40.6	41.5
9	51.0	50.4	51.4
10	37.4	37.2	37.9
11	20.7	20.9	21.7
12	27.5	27.2	27.4
13	34.3	37.5	38.9
14	40.7	42.2	43.1
15	28.3	28.3	30.5
16	22.3	31.4	32.6
17	51.2	56.2	56.7
18	55.1	49.2	50.2
19	79.3	51.2	43.6
20	–	212.2	156.5
21	29.7	29.7	33.0
22	29.0	36.7	37.3
23	28.1	28.0	28.5
24	15.4	15.3	16.1
25	16.5	16.1	16.6
26	15.8	16.0	16.5
27	13.3	14.7	15.0
28	179.3	180.4	177.5
29	–	–	106.3
30	–	30.1	64.7

made to prepare **4** through SeO_2 oxidation of **5** according to ref.[11]; thereby, **4** was obtained as a side product and siphoned off by CC. The ^{13}C NMR data of **3** and **4** are given in Table 1, detailed ^1H NMR data of **4** are listed in Table 2, where some assignments of the ref.[10] have been revised. Compounds **1–4** were also detected in the ether extract of *Leptospermum scoparium* Forst. wild plant material (source **II**) by TLC comparison (eluents 1 and 3) and RPTLC.

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Experimental

General

Optical rotation: Perkin Elmer 241.– IR: Perkin Elmer 298, KBr discs.– NMR: Bruker AMX500, standard Bruker software for HH-COSY, HMQC, HMBC experiments.– MS: Kratos MS50, EI, 70 eV.– TLC: Merck silica gel glass plates 60 F_{254}, 0.25 mm.– CC: Merck, a) Lobar RP-18 / B; b) open columns, silica gel 60 (63-200 µm).– Ozonisator Sander, type 301 (Eltze, FRG), 6.5 kV.

Plant Material, Extraction

Overground parts, a): source **I,** cultivated *L. s.* hybrids "type 7"[4], Stirnadel Company, Zweibrücken, FRG, 10 kg dried material, used for the isolation of **1–4**; b): source **II,** wild *Leptospermum scoparium* Forst. plants collected at Takapuna, Auckland, N.Z., in April 1994, 500 g dried material, used for TLC comparison. Extraction steps (**I** and **II**) as described in[4a]. Voucher specimens (**I** and **II**) are deposited in the Pharmazeutisches Institut der Universität Bonn, Kreuzbergweg 26, D-53115 Bonn, Germany.

Isolation of **1**

225 ml CH_3CN were added dropwise to a stirred soln. of 5 g ether extract[4a] in 25 ml $CHCl_3$ to yield 1.3 g amorphous precipitate. The latter, after washing with cold petrol ether, left a solid (0.55 g), which on CC sepn. (0.5 g; CH_2Cl_2, butanone, acetone, 92+4+4, = eluent 1; 120 g silica), gave **1** (11 mg, R_f: 0.29).

1, 3β,19β-dihydroxy-20,29,30-trinorlupan-28,19-olide: amorphous, off-white, mp 298 °C (acetone); (ref.[7]: 261–262 °C).– $[\alpha]_D^{20} = +22.5°$ ($c = 0.200$, EtOH). MS, [*m/z*] (rel. Int.): $HRM^{+\bullet}$: 414.3134 (1), calcd. for $C_{27}H_{42}O_3$: 414.6270; 397 (27); 396 (100); 381 (25); 353 (35); 314 (10); 189 (98).– IR $\tilde{\nu}$ [cm^{-1}]: 3380 br, 2950 s, 1765 vs, 1070. NMR data: see Tables 1, 2.

Isolation of **2–4**

10 g of the ether extract of **I**[4a] by CC (toluene, ethyl acetate, acetone, 80+10+10, = eluent 2) yielded 3.2 g of a crude triterpene fraction which was recrystallised from aqueous acetone (90%) to give **5** and ursolic acid (**6**), 1.8 g. The mother liquors gave 1.2 g solid (***A***), mainly **5** and **6**, with traces of **2–4**. Flash CC of ***A*** (2-chloropropane, acetone, ethyl acetate, 60+20+20, = eluent 3) yielded a subfraction with **2–4** (***B***, 85 mg). RP-18-CC of ***B*** (MeOH 85%) eluted **3** (6 mg), followed by **4** (10 mg) and **2** (32 mg) and was monitored by TLC (2-chloropropane, acetone, butanone, ethyl acetate, 40+20+ 20+20, = eluent 3; R_f values, **2**: 0.55; **3**: 0.47; **4**: 0.38–0.42).

2, 2α-hydroxyursolic acid: Mp: 236–242 °C (acetone; ref.[12b]: 241–245 °C); $[\alpha]_D^{20} = +42°$ ($c = 0.300$, EtOH; ref.[12b]: +49°), NMR shift values agree with published data[12a,b].

Table 2: ^{1}H NMR data of compounds **1** and **4**.

Proton	1 ($CDCl_3$)	1 (d_6 - benzene)	4 ($CDCl_3$)	4 (d_6 - acetone)
1-$H_{ax.}$	0.95 m	0.85 m	0.93 m	0.92 m
1-$H_{eq.}$	1.71	1.63	1.67 dm	1.65 ddd (12.8/7/3.5)
2-H_{AB}	1.55 – 1.66	1.53 – 1.65	1.50 – 1.64	1.52 – 1.60
3-H	3.19 ddbr (11.5/5/2)	3.14 ddm	3.22 dd	3.12 dd (11/5)
5-H	0.70 dm	0.69 dm	0.69 br	0.73 dm (10/3/1.5)
6-$H_{eq.}$	1.53 dm	?	1.52	1.53 dm
6-$H_{ax.}$	1.38 tm (2×10)	?	1.37	1.39 ddbr
7-H_{AB}	1.35 – 1.46	?	1.40 & ?	1.44 t & 1.36 m
9-H	1.27	1.26	?	1.33 dd
11-$H_{eq.}$	1.50	1.43	1.43	1.43
11-$H_{ax.}$	1.29	1.13 "q"d (3×12–13/4)	1.27	1.21 "q"br (3×13/4.5)
12-$H_{ax.}$	1.13 "q"br (3×13)	1.01 "q"d (3×13/4.5)	1.07	1.10 "q"br (3×13/4.5)
12-$H_{eq.}$	1.63 dm	1.47 ddd (12.5/9.5/3.8)	1.45	1.50
13-H	1.53	1.64	2.17 td	2.30 dddbr (13/12.5/3.5/1)
15-$H_{eq.}$	1.25	1.20	1.20 dm	1.17
15-$H_{ax.}$	1.52	1.82 tdbr (13.5/13/5/1)	1.53 ddm	1.53 m
16-$H_{ax.}$	1.53	1.37	1.43 ddm (12.5/3.5)	1.47 dm
16-$H_{eq.}$	2.06 m	2.28 dddbr (14.5/4.5/4/2)	2.29 dm (12.5)	2.24 dm
18-H	1.56 d	1.18 dbr (11)	1.74 t	1.76 t
19-H	4.62 brs	4.30 brs	2.88 td	2.91
21-H_A	1.87 – 1.90 AB	1.32	2.10 ddm (13/11.5/8.5)	1.99 tm
21-H_B	1.87 – 1.90 AB	1.62	1.42	1.39 dm
22-H_A	1.72	1.46	1.96 ddm	1.88 dd (13/8)
22-H_B	1.59	1.23	1.55 ddm	1.55
23	0.95 s	1.14 s	0.96 s	0.95 s
24	0.73 s	0.87 s	0.75 s	0.74 s
25	0.82 s	0.81 s	0.82 s	0.84 s
26	0.92 s	0.89 s	0.92 s	0.94 s
27	0.84 brs	0.76 s	0.98 s	1.01 s
29-H_A	–	–	4.96 brs	4.97 t (2×1.8)
29-H_B	–	–	4.92 brs	4.93 d (1.8)
30-H_2	–	–	4.13 brs	4.06 brs
3-OH	–	–	–	3.30 br
30-OH	–	–	–	3.75 t

Coupling constants in brackets. Protons listed without multiplicity are overlapped, their shift values are derived from *2D*-experiments.

3, platanic acid: Amorphous, white, mp 201–205 °C (acetone). ^{1}H NMR shift values are in agreement with published data [12a,b]. ^{13}C NMR data: see Table 1.

Methylation of **3**

Diazomethane treatment of 5 mg **3** in 20 ml diethyl ether yielded 5 mg **3**-methyl ester, mp 240–244 °C (ether, ref.[8]: 250–251 °C). $[\alpha]_D^{20} = -30°$ ($CHCl_3$, $c = 0.400$; ref.[8]: –30°).

Preparation of **3** *by Ozonisation of* **5**

A stream of ozone (~ 6% in oxygen, ~100 ml/min) was passed through a soln. of 20 mg **5** in 20 ml chloroform at room temp. On TLC monitoring, **3** appeared as the main spot (anisaldehyde-H_2SO_4 detection) next to **5** and two side products after three min, as described in ref.[8].

4, 3β,30-dihydroxy-lup-20(29)-en-28-oic acid: Mp: > 170 °C decomp. MS [*m/z*] (rel. Int.): HRM$^{+\bullet}$: 472.3562 (13), calcd. for $C_{30}H_{48}O_4$: 472.7068; 454 (22); 439 (10); 426 (8); 189 (100).
IR: $\tilde{\nu}$ [cm^{-1}]: 3430 s,br; 2940 vs; 1690 s. NMR data: see Tables 1,2.

Preparation of **4**

100 mg **5** upon 4h refluxing with 50 mg SeO_2 in 90% EtOH yielded, in correspondence to ref.[11], a mixture from which **4** was separated as a side product by CC (eluent 3; 8 mg).

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